# Expanded View Figures

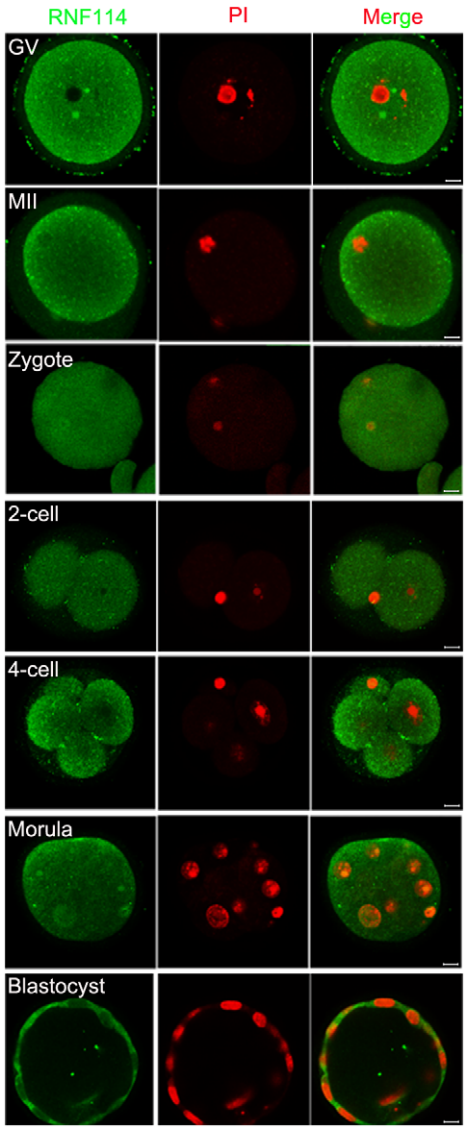


**Figure EV1. The expression of RNF114 during mouse early embryonic development.**
Immunofluorescence staining of RNF114 showed that the positive signals of RNF114 (green) are located in the cytoplasm from GV to blastocyst stages. PI nuclear staining (red). The results show that RNF114 was constantly expressed in the cytoplasm from GV oocytes to the blastocyst stages. Scale bar = 10 μm.

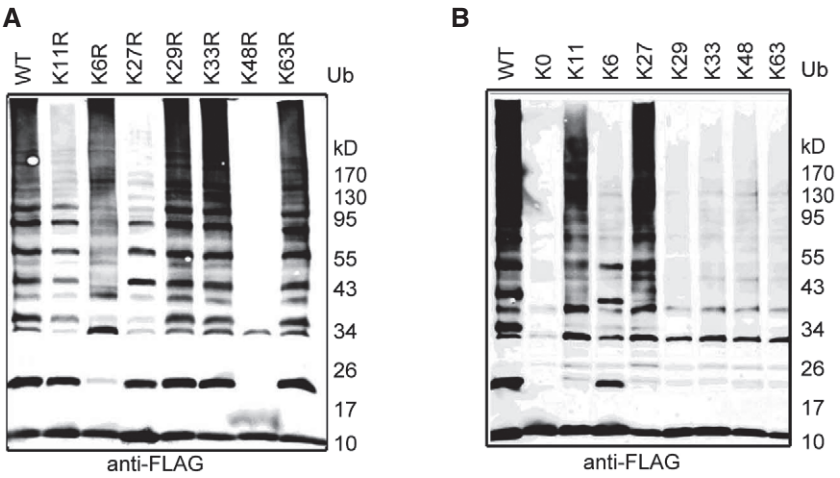


**Figure EV2. Linkage specificity of RNF114-mediated polyubiquitin chains.**

A, B Ubiquitin mutants (K63R, K48R, K33R, K29R, K27R, K11R, K6R, or K11 only, K6 only, K27 only, K29 only, K33 only, K48 only, K63 only, and K0) were used for ubiquitin chain assembly. K0 indicates that all seven internal lysine residues were mutated to arginine. In the absence of K6, K29, K33, or K63 residues (K6R, K29R, K33R, or K63R), the mutated ubiquitins could still be used to assemble polyubiquitin chains, while in the absence of K11, K27, or K48 (K11R, K27R, or K48R), the mutated ubiquitins could not be used to assemble polyubiquitin chains by RNF114. K11-only or K27-only ubiquitin could be used to assemble polyubiquitin chains by RNF114.

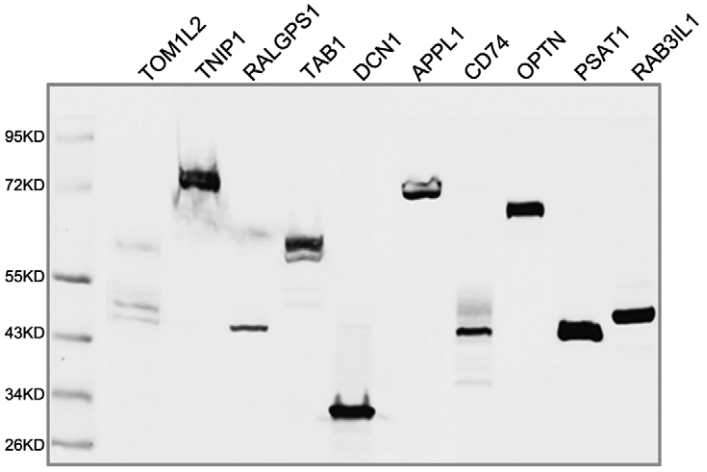


**Figure EV3. Eukaryotic expression of the potential substrate proteins.**

Ten FLAG-tagged candidate substrates were successfully expressed by HEK293T cells and detected by Western blotting.

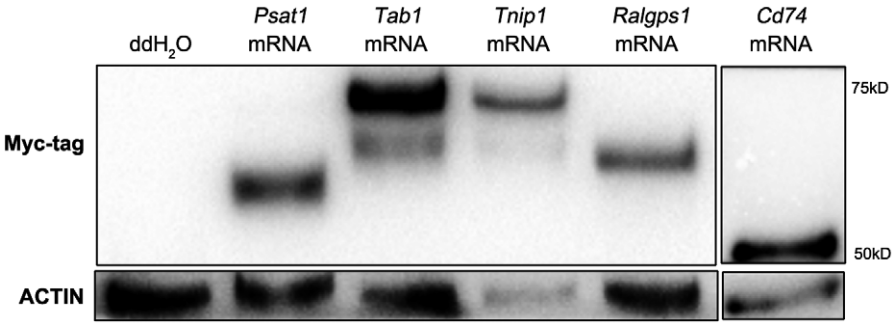


**Figure EV4. Overexpression of substrate proteins.**

RNase-free dd$H_2O$ (control group) or *in vitro*-transcribed mRNAs of myc-*Cd74*, myc-*Tab1*, myc-*Tnip1*, myc-*Psat1*, and myc-*Ralgps1* were microinjected into zygotes, and then, the expression of exogenous protein was detected by Western blotting using anti-myc tag antibody.

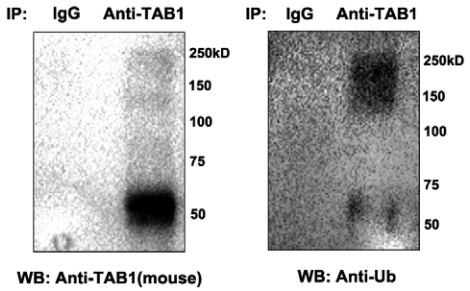


**Figure EV5. Endogenous ubiquitination of TAB1 in mouse two-cell-stage embryos.**

Cell lysates of mouse two-cell-stage embryos were immunoprecipitated by anti-TAB1 antibody, followed by Western blot analysis utilizing specific antibodies against TAB1 and Ub. A parallel immunoprecipitation with IgG was performed as a control.